Letters to the Editor . . .

MILK-BORNE CARCINOGENIC VIRUS

In 1936 it was shown by Bittner[1] that the apparently spontaneous mammary cancer in certain strains of mice is due to an "influence" or "incitor," transferred by nursing from the mother to the new-born young. Young born of the carcinophilic strain and foster-fed by mothers free from this apparently hereditary taint seldom if ever develop spontaneous breast cancer in late life. Young born from cancer-free strains and foster-fed by carcinophilic mothers often developed lethal breast cancer. It was afterwards shown that the same "incitor" may be demonstrated in either spontaneous or transplanted mammary cancer of mice[2] or in apparently normal lactating mammary tissues[3] of certain strains.

The active agent is present in cell-free filtrates from mammary carcinoma.[4] Tests were made on 4 or 5 week old females which had parents that did not transfer the influence in their milk but which had the inherited susceptibility for spontaneous mammary cancer. These young mice were inoculated intraperitoneally with Berkefeld filtrates from carcinoma tissues. In a typical group of 10 mice inoculated with such filtrates, 8 died and in a second group of 22 mice similarly inoculated, 12 died of spontaneous mammary cancer by the end of 12 months. The incidence of mammary cancer in several hundred uninoculated controls was less than 1 per cent.

These data suggest that the carcinogenic "influence" is a filtrable virus. If so, the "influence" presumably multiplies or is multiplied in symbiosis with mammary tissues. To test this possibility, 10 serial transplants of carcinoma tissue were made in mice that did not themselves carry the active milk agent. Berkefeld filtrates from the 10th serial passage caused the development of lethal mammary cancer in 8 out of 12 injected mice. There is thus evidence of the continuous production of the active agent within the transplanted tumor cells. Attempts to propagate the "influence" in embryonated hens eggs, thus far have not been very convincing, even though the milk agent survives for 12 days in the yolk sac in the absence of living mouse cells.

In order to obtain further evidence in support of the virus theory, Green[5] and his associates of the University of Minnesota studied the antigenic properties of the mouse-tumor agent. To do this, high-speed centrifugates from mouse mammary carcinoma filtrates were repeatedly injected into rabbits and white rats, both spontaneous mammary tumors and transplant tumors being used in making the filtrates. Control injections were made with filtrates from normal mouse tissues. Seven to ten days after the 5th injection serums were drawn from the injected animals, and tested for their possible virucidal action on the mouse-tumor agent.

To do this, centrifugate equivalents of 0.2 g. tumor tissue were suspended in 0.5 cc. saline solution and mixed with 0.18 cc. antiserum. After 2 hours standing at room temperature, 24 4-week old mice were injected with each mixture. Of 48 control mice injected with virus alone or with a mixture of virus and an antiserum against normal mouse tissues, 39 or 80 per cent developed breast tumors by the end of 13.5 months. Of 48 mice injected with a mixture of virus and specific antiserum (anti-milk "influence"), not a single case of breast tumor developed during the same period of time.

These findings confirm the earlier hypothesis that the milk agent is of exogenous origin. Whether or not the specific virucidal antiserum would be therapeutically effective in mice already infected with the milk-borne virus has not yet been tested.

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REFERENCES

1. Bittner, J. J.: Science, 84:162, 1936.
2. Bittner, J. J.: Science, 93:527, 1941.
Bulletin Minn. Med. Found., 4:94, 1944.
3. Bittner, J. J., Proc. Soc. Exp. Biol. and Med., 45:805, 1940.
4. Bittner, J. J., Cancer Res., 2:710, Science, 94:462, 1942.
5. Green, R. G., Moosey, M. M., and Bittner, J. J., Proc. Soc. Exp. Biol. and Med., 61:115 (Feb.), 1946.

PROTEIN-REPLETION THERAPY

In 1942 it was shown by Cannon[1] of the Department of Pathology, University of Chicago, that antibody formation is largely a function of protein reserves. Rabbits whose protein reserves had been reduced by plasmapheresis or prolonged low protein diets usually produced agglutinins of but one-fifth the titer of agglutinins produced by well-fed controls.

His implied theory of acquired immunity was of particular interest at that time due to its application to the epidemiology of infectious diseases under wartime conditions. Cannon[2] subsequently found that there is a positive correlation between protein deficiency and surgical infection. It therefore became of practical clinical interest to determine how promptly and effectively protein depletion can be corrected by dietary measures.

To test this[3] groups of adult white rats were placed on a protein depletion diet. The diet usually contained less than 2 per cent protein, with compensatory increases in non-protein factors so as not to reduce caloric intake. Vitamin intake was left constant. By the end of 83 to 191 days, there was 30 to 40 g. loss of body weight, and a 30 per cent reduction in hemoglobin and serum proteins. These depleted rats were then injected in the tail vein with washed sheep erythrocytes. They produced antiserums averaging 560 hemolytic units per cc. by the end of 6 days. As controls, normally fed rats were similarly injected. They produced antiserums averaging 560 hemolytic units per cc. by the end of 6 days. As controls, normally fed rats were similarly injected. They produced antiserums with an average hemolytic titer of about 8,000 units per cc. by the end of the same period, or 14 times the antibody titer of the depleted rats.

Protein-repletion tests were now made on other groups of depleted rats. To do this dehydrated beef, lacalbumin or proteinhydrolysate ("amigen") were added to the depletion diets in such a way as to increase the protein (or its equivalent) to about 20 per cent. There was practically no change in daily vitamins or caloric intake. Animals on these repletion diets made satisfactory weight recoveries (35-50 g. per rat) and serum protein regeneration (2.05 to 2.96 g. per cent) by the end of 7 days. During this 7-day recovery period, injection of washed sheep erythrocytes led to the production of antiserums of an average hemolytic titer of 3,830 units per cc. by the end of 6 days. This was nearly 7 times the average titer in depleted rats. A 2.6-fold improvement was seen after only 2 days protein-repletion feeding, increasing to